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Sir,

Capnocytophaga spp. have a role in the pathogenesis of various forms of periodontal disease and systemic infections, particularly severe in neutropenic cancer patients. The prevalence of β-lactam-resistant oral bacteria is increasing in clinical isolates [1]. All of the reported β-lactam-resistant *Capnocytophaga* isolates are β-lactamase-producers, but minimum inhibitory concentrations (MICs) for the different β-lactams are variable [2]. The objective of the current study was therefore

to explain the variability in β-lactam MIC profiles in 31 *cfxA* gene-positive oral *Capnocytophaga* spp. clinical isolates with various antibiotypes. This study investigated: (i) the presence of other β-lactamase genes in addition to *cfxA* (bla_{CSP-1}, *cepA*/*cblA* and *cfiA*); (ii) the expression level of *cfxA* in representative isolates with different antibiotic phenotypes; and (iii) the potential causes of *cfxA* expression variability, including mutation(s) in *cfxA* genes, location of the *cfxA* gene on a plasmid or the chromosome, and detection of the prevalence of mobile genetic determinants [*mobA*, *oriT*, *repA*, IS*Coc1* and transposons (Tn)] described as being involved in *cfxA* mobilisation and dissemination in *Capnocytophaga* spp. strains.

All 31 isolates were clearly identified as *Capnocytophaga gingivalis* (*n* = 1), *Capnocytophaga* spp. (*n* = 1), *Capnocytophaga ochracea* (*n* = 2), *Capnocytophaga granulosa* (*n* = 3), *Capnocytophaga leadbetteri* (*n* = 3), *Capnocytophaga* AHN9576/AHN9798/AHN8471/ChDc/ ChDCOS43 (*n* = 4) and *Capnocytophaga sputigena* (*n* = 17) by 16S rRNA gene sequencing. MIC_{90} and MIC_{50} values (MICs that inhibit 50% and 90% of the isolates, respectively) were all >256 mg/L for amoxicillin and first-and second-generation cephalosporins but were variable for third-generation cephalosporins. This variation in MICs for β-lactams was not due to the concomitant presence of other resistance genes: the *cepA*/*cblA* and c*fiA* genes were never detected, and the bla_{CSP-1} gene [3] was amplified in 11/31 (35%) of *cfxA*-positive *Capnocytophaga* isolates (Fig. 1). The presence of bla_{CSP-1} was not significantly associated with higher MICs of cefotaxime [MIC > 16 mg/L according to Clinical and Laboratory Standards Institute (CLSI) breakpoints (http://clsi.org/)] compared with the presence of *cfxA* only ($P > 0.1$). In four isolates, MICs of β-lactams were low (range, <0.016–2 mg/L) with a negative nitrocefin test, despite a

positive *cfxA* PCR. In 29/31 isolates, the presence of 966 bp corresponding to the complete sequence of *cfxA* was detected (27 isolates were β-lactam-resistant but 2 were β-lactam-susceptible). PCR assay, sequencing and in silico analysis showed that the CfxA COOH-terminal region (C-ter) in two susceptible isolates was replaced by a glycosyltransferase (96% homology) for one and with a partial hypothetical efflux pump (98% homology) for the other, with 16-bp and 82-bp overlapping gene sequences, respectively. Replacement of the whole C-ter region of *cfxA* was linked to β-lactamase gene inactivation, despite a positive *cfxA* PCR in the 5' region. Of note, the C-ter region of *cfxA* appeared to be a preferentially targeted area or 'hotspot' for the acquisition of foreign genetic material.

Among the clinical isolates, 52% harboured plasmids of 3.5, 5 and/or 9 kb. PCR amplified Tn (77.4%), IS*Coc1* (61.3%), *repA* (54.8%) and *mobA* (74.2%) that was related to plasmid detection ($P < 0.05$). This was not the case for the *oriT* gene (16.1%) ($P > 0.1$) (Supplementary Table S1). In the *cfxA*-positive *Capnocytophaga* isolates, *mobA and repA* genes were mainly detected (100% and 94%, respectively, among plasmid-positive isolates) and related to plasmid detection ($P < 0.05$). In 74% of *mobA*-positive isolates, the *mobA* and *cfxA* region were linked by a 96-bp intergenic sequence mainly found in plasmid-positive strains ($P < 0.01$). Higher MICs of cefotaxime (MIC > 4 mg/L) [Comite de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM) 2013; http://www.sfm-microbiologie.org/page/page/showpage/page_id/90.html] were significantly related to the presence of *mobA* ($P = 0.0002$), *mobA–cfxA* junction ($P = 0.0015$), *repA* ($P = 0.0003$) and at least one plasmid in bacterial strains ($P < 0.0001$). The presence of bla_{CSP-1}, *oriT*, *ISCoc1* or Tn did not significantly influence the MICs of cefotaxime.

bla$_{CSP-1}$ was detected on the chromosome or a plasmid and in different species of *Capnocytophaga*, indicating a large diffusion of this novel extended-spectrum β-lactamase among oral *Capnocytophaga* spp.

An unchanged complete *cfxA* sequence in two β-lactam-susceptible isolates indicated a possible misexpression of this gene under the influence of the *cfxA* genetic environment. Gene copy number as the only explanation of the expression variability of *cfxA* has already been observed [4].

The presence of the 96-bp junction *mobA*–*cfxA* sequence could be important for optimal expression of the *cfxA* gene. Higher MICs of β-lactams were related to *cfxA* overexpression arising from IS integration upstream of the coding sequence [5]. The diversity of plasmid profiles of *Capnocytophaga* isolates did not favour dissemination of several different β-lactam resistance genes via a single plasmid. In the current study, the *cfxA* gene could be located on a plasmid or/and the chromosome, whatever the species or MIC of β-lactams.

The numerous genetic rearrangements in the *cfxA* gene and the presence of various genetic mobile elements in oral *Capnocytophaga* suggest that this genetic site might be a reservoir for antibiotic resistance genes and their spread among different species. Furthermore, maintenance of this gene, even in an inactive form, might contribute to the persistence of antibiotic resistance genes in the oral flora.

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Fig. 1. Detection of *bla*$_{CSP-1}$, *mobA*, *cfxA*–*mobA* (junction), *oriT*, *repA*, transposons (Tn), IS*Coc1* and plasmids (presence of at least one plasmid) according to different species in clinical *cfxA*-positive *Capnocytophaga* isolates.

FIG 1. The *bla*$_{CSP-1}$, *mobA*, *cfxA-mobA (junction)*, *oriT*, *repA*, *Tn*, *ISCoc1* genes and plasmid detection (presence of at least one plasmid), according to different species in clinical *cfxA*-positive *Capnocytophaga* isolates.